

Destruction of *Staphylococcus aureus* During Frankfurter Processing

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We studied the thermal resistance of *Staphylococcus aureus* during frankfurter processing in respect to whether staphylococci are killed by the heating step of the process and whether heat injury interferes with the quantitative estimation of the survivors. With *S. aureus* 196E, heat injury could be demonstrated only when large numbers of cells (10^8 /g) were present and at a product temperature of 140°F (60°C). On tryptic soy agar and tryptic soy agar plus 7% NaCl media, at temperatures less than 140°F, the counts were virtually identical; above 140°F, the counts converged, with the organisms dying so rapidly that heat injury was not demonstrable. Heat injury was thus judged not to interfere with the quantitative estimation of staphylococci surviving the normal commercial heating given frankfurters. By using a combination of direct plating on tryptic soy agar and a most-probable-number technique, we detected no viable cells (<0.3/g) of several strains of *S. aureus* in frankfurters heated to 160°F (71.1°C). This temperature is compatible with the normal final temperature to which federally inspected processors heat their frankfurters and with the temperature needed to destroy salmonellae.

Staphylococci are part of the normal flora of hogs (17) and other meat animals (2). They are also part of the normal flora of man, residing in nasal passage, throat, and skin (2). Because of this ubiquitous occurrence in nature, they are often found in various raw meats (4, 7, 14, 16). Heiszler et al. (5) reported the presence of coagulase-positive staphylococci in all of 14 samples of frankfurter emulsion prepared in their pilot plant. The presence of staphylococci in raw meat and their known heat resistance (1) suggest that they could be a problem in heat-processed meat products. Though staphylococci are not usually isolated from most of these products, Jensen et al. (8) reported the presence of 7.5×10^3 coagulase-positive staphylococci per g in frankfurters. In addition, staphylococci often can be isolated from ham (9, 11).

One of the most important functions of the heating step of the frankfurter process is the destruction or reduction of the bacterial populations, especially those of public health significance and those that limit shelf life of the finished frankfurter. The study reported here is part of a continuing one to determine whether commercial processing procedures can produce pathogen-free cured meat products. We previously determined that heating frankfurters to 160°F (71.1°C) or above would produce a salmonella-free product (10). In this study, we in-

vestigated the effects of heat on staphylococci during frankfurter processing specifically in respect to (i) whether the heat injury phenomenon interferes with the quantitative estimation of staphylococci surviving various heat treatments, including the final one, and (ii) the thermal destruction of staphylococci during the heating step of the frankfurter process by quantitating the number of staphylococci surviving various heat treatments.

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MATERIALS AND METHODS

Emulsion. Standard frankfurter emulsion was prepared as described previously (10). The frankfurters were cured with a mixture of sodium ascorbate (0.529 g/kg of meat and fat) and sodium nitrite (0.154 g/kg of meat and fat). The emulsion was stuffed into 23-mm NOJAX casings (Union Carbide) and linked. The emulsion was prepared so as to yield finished frankfurters containing 30% fat.

Heating. The frankfurters were heated (with smoke) in an air-conditioned smokehouse according to the standard step schedule shown in Table 1. Internal temperature of the frankfurters was monitored continuously during heating by a thermocouple inserted into the center (along the longitudinal axis) of a cooked frankfurter and was recorded with an Elektronik model 16 recorder (Honeywell). Samples of staphylococci-containing frankfurters were removed

TABLE 1. Standard step schedule used in heating of frankfurters

Bulb	Temp [°F (°C)] at:			
	10 min	30 min	45 min	"a"
Dry	130 (54.4)	145 (62.8)	165 (73.9)	190 (87.8)
Wet		135 (57.2)	140 (60.0)	170 (76.7)

^a Held at this setting until the product reached the final temperature, generally 160°F (71.1°C). The time to reach this final temperature was 95 ± 5 min.

from the smokehouse as the product reached various predetermined temperatures/times or heat treatments. Heat treatment or degree-minute (deg-min) for each sample was determined by an integration procedure described previously (10, 12).

Preparation of staphylococci-containing emulsion. A 24-h broth culture of *Staphylococcus aureus* (10-ml tube of tryptic soy broth [TSB; Difco] grown at 35°C served as inoculum for most experiments. Appropriate quantities of this culture were added to preformed emulsion along with green food dye as described previously (10). When large quantities of cells were needed, the 24-h broth culture was used to inoculate a series of 200-ml quantities of TSB (contained in 1,000-ml Erlenmeyer flasks). These flasks were incubated for 24 h at 35°C with shaking (200 rpm). These shaken cultures were then centrifuged (10 min, 10,000 × g, 2°C), pooled, and suspended in ca. 20 ml of 0.1% peptone (Difco) water and 3 ml of green food dye and added to the preformed emulsion. In one experiment, the culture and food dye were added directly to the Schnell cutter and incorporated into the emulsion.

Strains of *S. aureus*. The following strains of *S. aureus* were used: 196E, 184, NRRL B-313, 18132, 1342-14A, and MF 31.

Enumeration of surviving organisms. (i) **Direct plate count.** Fifty grams of emulsion or heated frankfurter was immediately weighed aseptically into a cold, sterile Waring blender jar, 200 ml of cold peptone water was added, and the mixture was blended for 1 min at high speed.

Survivors of the various heat treatments were quantitated by surface-plating dilutions of the blended emulsion in triplicate onto tryptic soy agar (TSA; Difco) and TSA + 7% NaCl (TSAS). Because of the high numbers of *S. aureus* added, survivors of the natural microflora were usually not encountered. Further, since all strains of *S. aureus* used formed golden colonies, they could easily be differentiated and counted on the plating media. The plates were counted after 48 h of incubation at 37°C. The number of heat-injured cells at any heat treatment is the difference in count between TSA and TSAS. The lower limit of detection (LLD) by the plate-count procedure was 50/g.

(ii) **MPN method.** The following three-tube most-probable-number (MPN) procedure was developed to quantitate low numbers of surviving *S. aureus*. The LLD of the MPN procedure was 0.3/g. Appropriate dilutions of the blended emulsion were inoculated into tubes of brain heart infusion (Difco) broth and incubated for 48 h at 37°C. Growth from each MPN tube

was then streaked onto Baird-Parker agar (Difco) plates and incubated for 48 h at 37°C. Shiny black colonies that were lecithinase positive were picked into 0.2 ml of TSB and incubated for 24 h at 37°C. Coagulase plasma (Difco: 0.2 ml) was then added to the TSB culture and incubated at 37°C. These tubes were observed periodically for clot formation; isolates that coagulated the plasma within 8 h were considered coagulase positive. The original black colony was also tested for catalase and Gram reaction. Shiny black colonies of gram-positive cocci that were positive for coagulase, catalase, and lecithinase were considered to be *S. aureus*.

RESULTS

Heat injury. The results of early experiments indicated that heat injury of *S. aureus* 196E was not readily demonstrable when the viable cell count per gram of emulsion was 10⁸ cells or less. To determine the presence of heat-injured cells, we employed the TSA-TSAS plating system (13).

The results of a typical experiment in which >10⁸ cells of *S. aureus* 196E per g of frankfurter emulsion were used are presented in Fig. 1. Heat injury was best demonstrated at ca. 1 deg-min (temperature range of 140 to 144°F [60 to 62°C]); at <1 deg-min, the counts on TSA and TSAS were similar, whereas at >1 deg-min, the cells were killed. The use of a salt-containing medium could give misleading information regarding the ability of the heating step to kill staphylococci if a frankfurter were heated to only 140°F. However, in our process and in most commercial ones (15), the product is heated to ca. 160°F, which is equivalent to a thermal exposure of ca. 2.3 deg-min. The counts on TSA and TSAS converged above 1 deg-min. The counts on TSAS tended to reach the LLD sooner than the counts on TSA, but the total heat treatment resulted in the complete destruction of staphylococci regardless of the detection medium (Table 2, Fig. 2).

In another experiment studying heat injury,

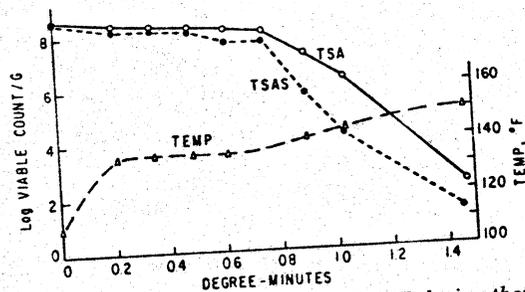


FIG. 1. Heat injury of *S. aureus* 196E during thermal processing of frankfurters; plated on TSA (total count) and TSAS (noninjured cells).

TABLE 2. Temperatures at which viable *S. aureus* are and are not detected during thermal processing of frankfurters

Strain	Temp [°F (°C)] at which viable cells are detected	MPN/g at that temp	Temp [°F (°C)] at which no viable cells ^a are detected
196E	150 (65.6)	2.3	155 (68.3)
184	152 (66.7)	0.9	160 (71.1)
NRRL B-313	152 (66.7)	4	160 (71.1)
1342-14A	154 (67.8)	0.9	160 (71.1)
18132	152 (66.7)	0.9	160 (71.1)

^a MPN < 0.3/g.

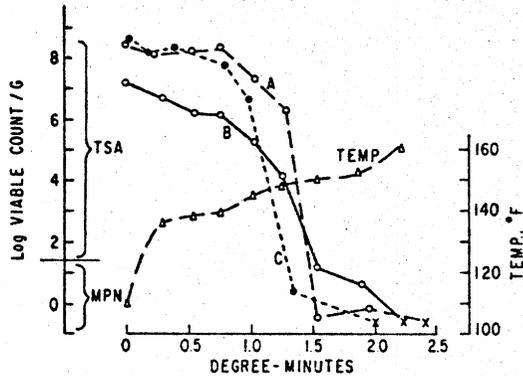


FIG. 2. Destruction of various strains of *S. aureus* during frankfurter processing. ×, Count at or below the LLD. A, Strains 184, 1342-14A, and 18132; B, strain NRRL B-313; and C, strain 196E.

the thermal response of *S. aureus* MF 31 at a level of $>10^6$ /g of frankfurter emulsion was investigated by plating samples of the heated frankfurters on TSA and TSAS. Data similar to those for *S. aureus* 196E (Fig. 1) were obtained. However, for this organism, the greatest amount of heat injury (largest difference in count) occurred at a thermal exposure of 1 deg-min (148°F; 64.6°C).

Total process. The ability of the total heat treatment of the frankfurter process to kill staphylococci was evaluated with different strains of *S. aureus* by a combination of the direct-plating (on TSA) and MPN methods (Fig. 2). The viable *S. aureus* counts for the high-temperature-high-heat treatment (deg-min) portion of Fig. 2 are presented in Table 2. These data indicate that heating of frankfurters to 160°F insures production of *S. aureus*-free products.

No data on the killing of *S. aureus* MF 31 during frankfurter processing are presented. Inconsistent behavior of this organism during thermal destruction studies led us to believe that there are different temperatures to which frankfurters should be heated to be free of *S. aureus*

MF 31. Data for *S. aureus* MF 31 were similar to that of other strains (Fig. 2) except that the "tail-off," the lower portion of the viable count plot, started at log 2, and viable cells were often detected at 3 deg-min (170°F; 76.7°C). This occurred even with a low starting count. However, in one experiment, *S. aureus* MF 31 died off completely in frankfurters heated to only 150°F (65.6°C; ca. 2 deg-min). Stiles (Ph.D. thesis, University of Illinois, Urbana, 1963) stated that *S. aureus* MF 31 had the highest $D_{130^\circ\text{F}} (54.4^\circ\text{C})$ of any strain he tested and that thermal death time plot was linear (no shoulder or tail-off). In contrast, we observed that the slope of the central portion of the log viable count versus deg-min for *S. aureus* MF 31 was similar to that of the other strains and that it differed only in this "tailing-off" phenomenon.

Fast house. The purpose of this study was to determine how rapidly *S. aureus* would die during the frankfurter process and whether the standard process or a rapid attainment of high temperatures was more lethal to *S. aureus*. For this experiment, the emulsion containing *S. aureus* 196E was heated in a smokehouse set at 190°F (87.8°C; dry bulb) and 170°F (wet bulb) instead of the standard step schedule settings. The response of the organism thus processed is given in Fig. 3. Heat injury was best demonstrated at 138 to 143°F (58.9 to 61.7°C; 0.28 to 0.41 deg-min). Below this heat treatment, there was little heat injury or killing; above this, the cells died so rapidly that it was not possible to demonstrate any injury. The count of *S. aureus* reached the LLD at a lower heat treatment (deg-min) than in any other experiment. The frankfurters produced by using this fast-heating schedule, especially those heated to only 0.4 deg-min, were soft in texture and atypical in appearance.

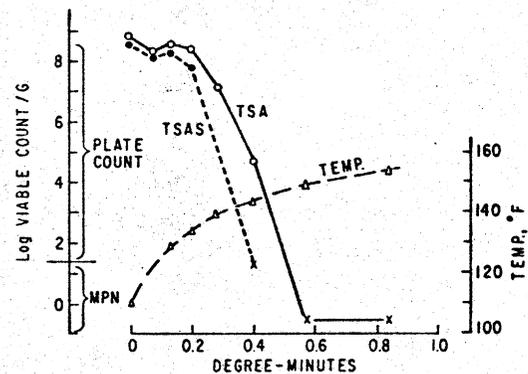


FIG. 3. Destruction of *S. aureus* 196E during thermal processing of frankfurters in a fast smokehouse (set at 190°F dry bulb and 170°F wet bulb). ×, Count at or below the LLD.

Incorporation into the emulsion. A pronounced shoulder on the survivor curves (Fig. 1 and 2) indicated relatively little killing of *S. aureus* below 0.8 deg-min (140°F). This could have resulted from clumping of the cells during natural growth of the culture or in the centrifugation step. This effect was investigated by comparing the killing of *S. aureus* 196E centrifuged and added to preformed emulsion with the killing of the organism incorporated directly into the emulsion as culture broth without centrifugation. The survivor plots for the two emulsions were similar to the data of Fig. 1 and 3 and indicated that: (i) the shoulder is not caused by the centrifugation step, and (ii) incorporation of the organism into the emulsion did not offer any increased protection to thermal destruction. Since all strains of *S. aureus* we tested clumped to some extent, it is not possible to determine whether the shoulder of the survivor curves was due to natural clumping of the organism or their pattern of thermal resistance.

Growth in raw emulsion. The potential for growth of *S. aureus* in raw frankfurter emulsion was investigated by inoculating the emulsion with the organisms, stuffing it, incubating it at 5, 20, and 37°C, and counting samples at intervals (Table 3). At 5°C, the count decreased, whereas at both higher temperatures, significant growth occurred even after 1 day of incubation.

DISCUSSION

The data presented here on the thermal destruction of *S. aureus* during frankfurter processing provide support for and confirmation of the observations of Surkiewicz et al. (15) that frankfurters are staphylococci-free after being processed and before being peeled. Surkiewicz et al. (15), in several commercial plants, observed that most frankfurters produced under federal inspection are heated to an internal temperature of 69 to 71.1°C (158 to 160°F), the lowest temperature observed being 66°C (150.8°F) and the highest 82°C (179.6°F). They determined that all 127 samples of cooked frankfurters were *S. aureus*-free before being peeled and packaged. Thus, the temperature used by most frankfurter processors is sufficient to kill very large numbers of *S. aureus* in frankfurter emulsion. Hill (6) stated that cooked-meat products such as bologna and frankfurters should be staphylococci-free. His contention, supported by Surkiewicz et al. (15), was that any staphylococci found on cooked-meat products result from post-processing contamination; our data also support this idea.

We found that, at least for the strains used and for frankfurters cooked according to the

TABLE 3. Fate of *S. aureus* 196E in raw frankfurter emulsion held at different temperatures

Days	Count ^a of raw emulsion ($\times 10^8$) at:—		
	5°C	20°C	37°C
0	14	14	14
1	ND ^b	3,300	1,900
2	6.8	ND	2,300
3	ND	2,000	2,300
4	4.6	1,800	ND

^a Surface plated on TSA and counted after 48 h at 37°C.

^b ND, Not done.

step schedule described, heating frankfurters to 150°F is inadequate or at best marginal to destroy all viable *S. aureus* (Fig. 1 and 2, Table 2). This agrees with the findings of Castellani et al. (3) that heating to at least 154°F (67.8°C) was required to destroy all viable staphylococci in turkey stuffing during roasting.

Somewhat anomalous behavior was observed when frankfurters containing *S. aureus* 196E were heated in a fast house (Fig. 3). In this instance, the 6×10^8 organisms per g died by the time product temperature reached 148°F (0.57 deg-min); both indexes of heat treatment, deg-min and temperature, were considerably lower than that required for destruction in other experiments (Fig. 2, Table 2). Most data on killing of bacteria indicate that thermal destruction is a time-temperature relationship. However, the decimal reduction time data (*D* values) of Stiles and Witter (13) and Angelotti et al. (1) indicate a nonlinear relation between temperature and *D* value: the higher temperatures are much more lethal than the lower ones. For example, Angelotti et al. (1) reported *D* values for *S. aureus* 196E heated in "chicken à la king" of 61, 20, 6.7, 2.2, and 0.64 min for 130, 135 (57.2°C), 140, 145 (62.8°C), and 150°F, respectively. The rapid attainment of the higher and thus more lethal temperatures probably contributed to the more rapid destruction of *S. aureus*.

The standard step heating schedule described is necessary to form the skin of a skinless frankfurter and to set the emulsion. The destruction of salmonellae (10) and staphylococci (this work) occurred concomitantly with the physical formation of the frankfurter. In terms of destruction of staphylococci, a fast house is adequate. However, the frankfurters produced were softer than those from a regular process, and their skin was less firm. In addition, informal sensory evaluation indicated that these rapidly processed frankfurters were softer in texture, and, because they were exposed to a shorter smoke period (50 min instead of the usual 95 min), their flavor

was not as desirable as that of the normally processed frankfurters.

Concern is often voiced that the usual selective procedures employed to isolate various pathogens from foods will allow the enumeration of organisms sublethally injured by processing operations such as heating or freezing. In this study, heat injury of *S. aureus* was demonstrated, but only with very large numbers of cells ($>10^8$ /g) and only at certain portions of the heating cycle (ca. 140°F). If frankfurters were heated at 140°F, the use of a salt-containing medium such as mannitol salt agar could give a misleading picture of the effectiveness of the frankfurter thermal process against *S. aureus* (Fig. 1). In addition to the fact that frankfurters are heated to temperatures above 140°F, typically 158 to 160°F (70.0 to 71.1°C) (15), the counts on TSA and TSAS converge above and below 140°F. Thus, while heat injury of *S. aureus* could be demonstrated during frankfurter processing, it did not appear to interfere with quantitation of organisms surviving the final processing temperature. Further, the view is generally held that any *S. aureus* isolated from a cooked-meat product would result from post-processing contamination, and, thus, heat injury should not interfere with quantitation of these nonheated organisms (6, 15).

Heat injury could, however, interfere with quantitation of surviving *S. aureus* in other meat products such as bacon, which is heated to temperatures not exceeding 128°F (53.3°C) or various pork-containing sausages, i.e., summer sausage, which are given only a "trichina cook" (heated to a maximum of 137 to 140°F [58.3 to 60.0°C]).

The rapid growth of *S. aureus* 196E in raw emulsion (Table 3) suggests that the emulsion should be heat processed as soon as possible after preparation. The nitrite (starting level of 0.154 g/kg drops to ca. 0.100 g/kg very soon after preparation of the emulsion) and salt concentration (2.5%) is not sufficient to deter the growth of *S. aureus* 196E. Though we demonstrated that the heating step of the frankfurter process can reduce a starting staphylococcal count of $>10^8$ /g to undetectable levels, temperature abuse of raw emulsion should not be condoned because of the possible formation of heat-stable enterotoxin by *S. aureus*.

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